The Future of CRISPR/Cas9: Ensuring Safe and Ethical Human Gene Editing

Joshua M. Brenner

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Steven A. Vokes, Ph.D.
Institute for Cellular and Molecular Biology
Supervising Professor

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Kenneth I. Shine, M.D.
Dell Medical School
Second Reader

Abstract

Author: Joshua Brenner

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Supervising Professor: Steven A. Vokes, Ph.D.

CRISPR/Cas9 is a relatively recent development in biotechnology that makes it simpler than ever before to add and remove genes from groups of cells. Most treatments currently in development target specific populations of somatic cells, and as such are not heritable and are relatively limited in their long-term effects. However, efforts to genetically modify human embryos have also recently taken place—if successful, these changes would affect the entire human cell population and be fully heritable. Such modifications introduce a host of social and ethical issues and lend validity to concerns that gene editing could be adapted for eugenics. This paper addresses the danger that germ-line editing technology poses and examines potential difficulties facing regulators who may hope to ameliorate its effects.

Ultimately scientists should attempt to develop an internationally standardized agreement outlining what kinds of gene editing experiments are and are not permitted; until this is possible, they should try to achieve an informal general consensus through regular international conferences and encourage regulation that mirrors this consensus wherever possible.

Introduction

Almost since the discovery of DNA as a heritable genetic element, scientists and non-scientists alike have discussed the possibility that we might one day be able to alter our genetic fates to suit our preference. To some, this technology holds the potential to herald a new era of medicine where the genetic cause of disease can be addressed before symptoms ever have the opportunity to manifest. Others fearfully speculated that gene editing would produce a reinvigoration of eugenics, and that the technology might be utilized to artificially reinforce pre-existing class structures and biases.

Over the past quarter of a century, genome editing has shifted increasingly from the realm of science fiction to a reality. This has become particularly true with the recent advent of CRISPR/Cas9, a protein/RNA complex that makes altering the genetic makeup of cells to suit a wide variety of needs trivially simple. Early clinical trials and even a handful of clinic-ready treatments have made it clear that CRISPR/Cas9 is a technology with extraordinary therapeutic potential. However, recent attempts to use CRISPR/Cas9 to edit the genomes of entire embryos have raised concerns about the potential long term social and health-related consequences that mastery of gene editing techniques might carry with them. Germ-line genetic changes are heritable, after all, and could have drastic and unforeseeable effects on future generations.

In this essay, I will argue that the potential ethical hazards posed by germ-line genome editing outweigh any potential clinical benefits it might provide. I intend to outline a regulatory approach through which this harm could be mitigated; although achieving consistent scientific regulations across national boundaries is not as

straightforward as it once was, efforts to do so with regards to gene editing are clearly in global human interests. To guide my recommendations, I will examine past and contemporary attempts to develop effective regulatory policies to determine how these approaches can be applied to the unique challenges posed by gene editing in the modern era. Many prospective applications of genome editing have the potential to vastly improve human health, however scientists and regulators must carefully weigh their unintended consequences in a mosaic of global regulatory environments to determine which are and are not worthwhile pursuits.

Chapter I – The Future of Gene Editing

Clustered regularly interspaced short palindromic repeats, or CRISPRs, were first characterized by a group from the University of Osaka led by Yoshizumi Ishino, when he noticed that a bacterial DNA sequence he was studying was flanked by an unusual repetitive sequence[1]. These sequences were later discovered to have a role in the bacterial immune system as part of a complex, CRISPR/Cas9 that would recognize foreign DNA (presumably injected by viruses) and cut it into pieces which could then be relegated to a subcellular compartment and/or destroyed[2]. Because of the system's ability to recognize specific DNA sequences and to produce double stranded breaks in precise locations, CRISPR/Cas9 quickly gained recognition as a potential tool for genome editing.

Researchers developing genome editing treatments must first identify a DNA sequence of interest in their target cell and design a CRISPR RNA complimentary to some portion of that sequence. They can then introduce CRISPR-coupled Cas9 system into cells, where it will be directed to produce a double-stranded break onto a specific region of host DNA like a pair of scissors snipping through a thread[3]. In non-homologous end joining (NHEJ), this cutting action can be used to simply remove a sequence of DNA. This high-efficiency process usually introduces some number of random base insertions

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[1] Ishino Y, Shinagawa H, Makino K, Amemura M, Nakata A. "Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product". *Journal of Bacteriology,* 1987.

[2] Mojica FJ, Díez-Villaseñor C, García-Martínez J, Soria E "Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements". *Journal of Molecular Evolution*, 2005

[3] Marraffini, Luciano A., and Erik J. Sontheimer. "CRISPR Interference: RNA-directed Adaptive Immunity in Bacteria and Archaea." Nature Reviews. Genetics. March 2010.

or deletions between the two ends, termed "indels[4]." The propensity for CRISPR/Cas9 to produce indels has been a major source of concern to researchers and regulator due to the relatively low fidelity of the system—it has the tendency to bind at off-target sites and then to introduce mutations into a random part of the genome. This random binding activity could then lead to further disease[5]. If coupled with the introduction of another DNA sequence with homologous ends, CRISPR/Cas9 can also be used to add in a new piece of DNA to the targeted genome using homology-directed repair (HDR)[6].

Although various treatment strategies using CRISPR/Cas9 has already been introduced in a number of settings, its current technical limitations—namely, issues with fidelity and efficiency—have somewhat reduced the number of prospective options that are ready for use. However, some pioneers in the field have developed treatments using CRISPR/Cas9 systems that are able to circumvent these limitations in some way. These frequently involve applying the treatment to a relatively small number of cells and introducing mechanisms to control cell survival and proliferation[7]. This helps to ensure that if off target mutations do occur and produce a diseased state in the selected cells, that the damage done is limited. Others actually take advantage of the indel-inducing

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[4] Daniel P. Dever et al. CRISPR/Cas9 β-globin gene targeting in human haematopoietic stem cells. Nature. 2016 Nov 7

[5] Sander, J. D. & Joung, J. K. CRISPR–Cas systems for editing, regulating and targeting genomes. *Nature Biotechnol.* **32**, 347–355 (2014)

[6] Chu, Van Trung. "Increasing the Efficiency of Homology-directed Repair for CRISPR-Cas9-induced Precise Gene Editing in Mammalian Cells." *Nature Biotechnology* (2015

[7] Rooney, Cliona. "Current Uses of CRISPR/Cas9 in the Baylor College of Medicine Center for Cell and Gene Therapy." Interview w/ Joshua Brenner, March 2017.

properties of cas9, intentionally damaging the functionality of target genes in an effort to alter protein expression[8].

The first clinical trial using CRISPR/Cas in humans began in China in October of 2016, led by Dr. Lu You at the Sichuan Univervisty[9]. This trial targets multiple genes in mature T cells that will hopefully aid in their ability to combat cancer in late stage cancer patients. The treatment involves the insertion of a gene for a receptor that will allow the T cells to recognize an antigen, NY-ESO-1, that is commonly presented by lung cancer cells. Researchers will also a gene that down-regulates T cell activity called PD1[10].The former will allow T cells to target NY-ESO-1-expressing cells so that they can attack them directly, hopefully reducing numbers of cancer cells present in the patient; the latter should cause the T cells to be up regulated, improving their survivability and increasing their activity. Together, the modifications are expected to improve patient outcome.

Dr. You's group has taken a multi-faceted approach to minimize the risks discussed above. First, they will use a high-fidelity variant of Cas9 that will help to minimize off target effects. They will also perform genetic screens on the T-cells to determine whether or not any off-target sites have been affected, possibly inducing cancer-causing mutations. Finally, the protocol is only approved in patients who have

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[8] Daniel P. Dever et al. "CRISPR/Cas9 β-globin gene targeting in human haematopoietic stem cells." Nov 2016.
[9] Cyranoski, David. "CRISPR Gene-editing Tested in a Person for the First Time." *Nature News*. Nature Publishing Group, November 2016.
[10] Ibid

fewer than six months to live[11]. These patients have undergone all other standard treatments and have continued to progress in their disease. If there are unforeseen complications with the therapy, this final safeguard will ensure that the suffering caused is less grievous than if it were used in healthier people. The trial is hoped to both improve patient outcomes, and provide a proof-of-concept—since it is the first time that CRISPR/Cas9 has been used in humans, it will be useful to have evidence showing whether or not it works as anticipated. This is an important first step that will hopefully help to clear the way for the use of CRISPR treatments in people who are less seriously ill.

T cell therapies using CRISPR systems are also being developed in the US, although they typically face slower and more onerous regulatory processes before they can be used in patients. A group of labs led by Dr. Cliona Rooney at Baylor College of Medicine is developing T cells that have been genetically modified to recognize tumor antigens and improve cell survivability. They also hope to include as a kind of safety feature to the cells, a "suicide switch." They have used CRISPR/Cas9 to alter a gene that makes their modified T cells extremely vulnerable to a certain drug that should leave unmodified cells unaffected. As such, if there are unexpected side effects following administration of the cells to a patient—if the cells start attacking the wrong targets, or if off-target effects from the CRISPR/Cas9 treatment cause the cells to behave erratically—

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[11] Lu, You. PD-1 Knockout Engineered T Cells for Metastatic Non-small Cell Lung Cancer. Clinical Trials US Gov. Database. Sichuan University. November 2016.

clinicians can simply administer the drug and kill off the modified cells. In the near future, Dr. Rooney's lab hopes to use these "CAR" (chimeric antigen receptor) T cells to improve survival in patients with certain types of cancer[12].

People with monogenic disorders, diseases caused by a malfunction in a single gene rather than by contributions from a number of genes, are excellent prospective candidates for CRISPR/Cas9 treatments. The best non-genetic treatments available will, by definition, treat only the symptoms of these disorders rather than their cause. With the recent improvements to gene editing brought by CRISPR/Cas9, a greater and greater number of researchers are looking into potential mechanisms to directly repair the genome of patient cells, which is a much longer term solution than currently available treatments.

These monogenic diseases include conditions such as ß-thalassemia, an autosomal recessive genetic blood disorder in which functional ß hemoglobin subunits are not produced in sufficient numbers. As a result, less functional hemoglobin is present in red blood cells than in healthy people, meaning that their ability to deliver oxygen to organs and muscles is greatly reduced[13]. Hemoglobin diseases such as ß thalassemia are particularly well suited to CRISPR/Cas treatment because of the existence of γ-globin. γ-globin is a hemoglobin chain that is normally expressed in fetuses and very young babies. Long before adulthood, it is repressed and replaced with ß-globin. In individuals

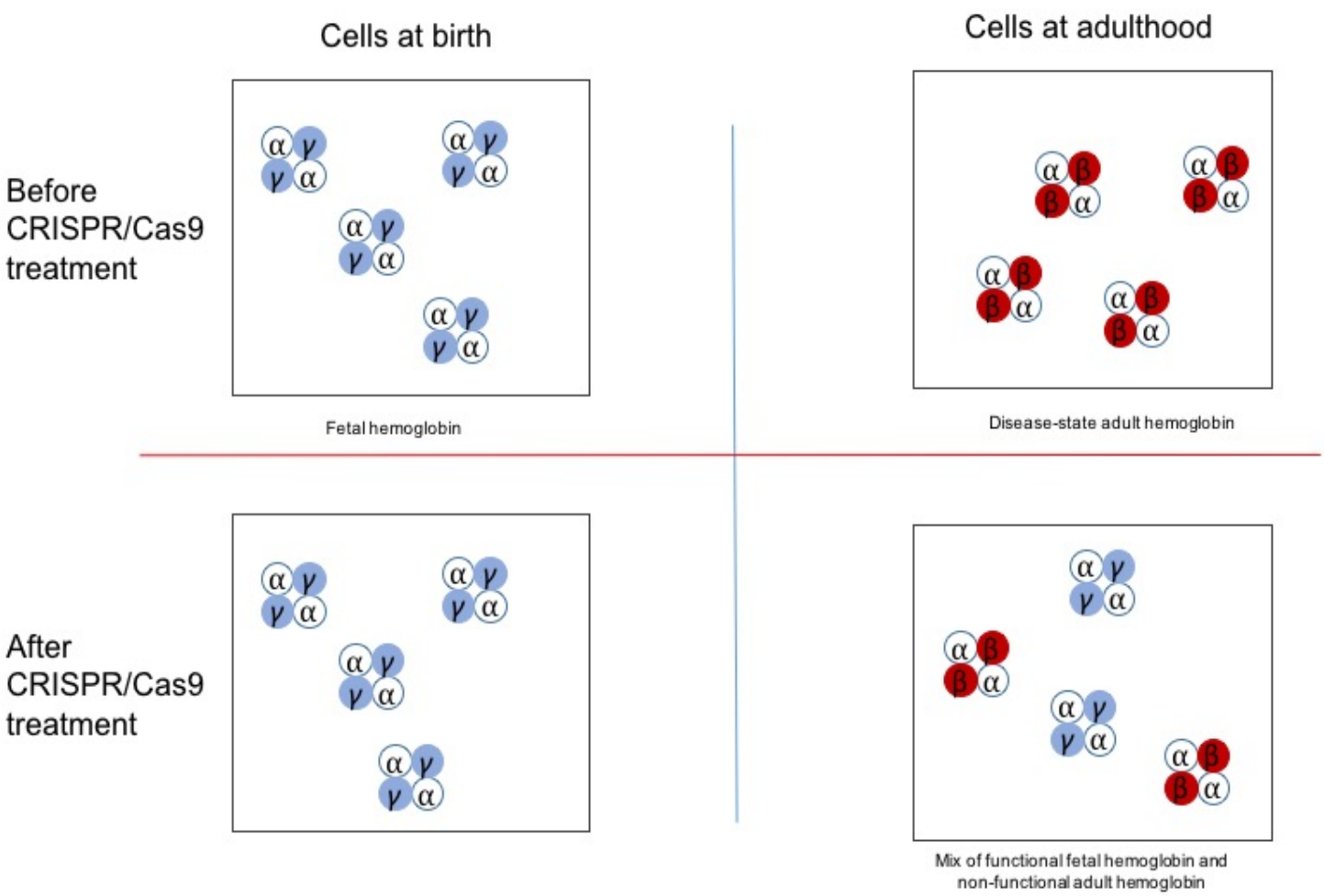
[12] Appendix I, Dr. Cliona Rooney, "Current Uses of CRISPR/Cas9 in the Baylor College of Medicine Center for Cell and Gene Therapy." March 2017.
[13] Origa R. Beta-Thalassemia (Review Article). 2000 Sep 28, updated 2015.

with a faulty copy of ß-globin, this initiates the disease state[14]. One promising treatment strategy, then, would be to attempt to somehow induce a return to the expression of fetal hemoglobin and repression of adult hemoglobin in people with ß thalassemia, allowing them to produce healthy blood cells and relieving the symptoms of their anemia.

A group of researchers at St. Jude, led by Dr. Elizabeth Traxler, has attempted to do just that. Dr. Traxler's lab removed hematopoietic blood cells from patients with ß-thalassemia and Sickle Cell anemia, intending to edit their genomes to cause them to express fetal hemoglobin. They used a variant of cas9 that is known to be very prone to producing random indel mutations and used it to disrupt a repressor of γ-globin transcription. This caused an increase in the number of γ-chain proteins present in the cell. This novel approach actually utilizes one of the major drawbacks of CRISPR/cas9—its tendency to insert and delete random nucleotides at the locations where it cuts—as a treatment strategy. This treatment, or one of a number of similar approaches, could soon be ready for use in clinical trials[15].

[14] Edoh D, Antwi-Bosaiko C, Amuzu D. Fetal hemoglobin during infancy and in sickle cell adults. Afr Health Sci. 2006

[15] Elizabeth A. Traxler, et al. "A genome-editing strategy to treat β-hemoglobinopathies that recapitulates a mutation associated with a benign genetic condition." Nat Med. 2016



Fetal globin chain expression can be induced via lentiviral vector delivery of CRISPR/Cas9 in hematopoietic blood cells, which are then introduced into the patient. Over time, the stem cells proliferate and differentiate into red blood cells with functional hemoglobin, which could reduce symptoms over a long period of time in patients with hemoglobin disorders like ß thalassemia sickle cell anemia.

The vast majority of research resources devoted to CRISPR/Cas9 have been aimed at developing treatments similar to those described above. These treatments are classified as “somatic cell” treatments, because they target either fully differentiated cells or stem cells that are not totipotent (that is, they are not able to give rise to an entire embryo). Such treatments target genes in a single cell type or tissue in an effort to restore the patient from the disease state. However, some genetic disorders affect a variety of cell types, cell types that are, for whatever reason, not readily accessible to CRISPR/cas9, or else are so deadly that by the time medical intervention using somatic cell gene editing treatment would be possible the damage would be done. This has raised interest with some groups in the possibility of directly editing human germ-line cells—that is, cells that will develop into an embryo and eventually into every cell type in the body.

The first study to ever attempt to genetically modify human germline cells was carried out by a Chinese group led by Junjiu Huang published in May 2015. His group used around 80 human embryos, all of which were inviable products of IVF that had been donated for research purposes. They used CRISPR/Cas9 to target a gene for ß-hemoglobin, splicing out a small segment of the gene and attempting to replace it with a new sequence through homology dependent recombination. This approach has some similarity to gene editing treatments used to combat ß-thalassemia, though obviously these treatments occur in hematopoietic blood cells which are a somatic cell line. Huang et al found that fewer than half of the embryos that he had treated were missing the

gene segment that the CRISPR had targeted. Of these, only a small number had successfully incorporated the newly introduced sequence. Because the embryos were allowed to develop during the treatment, of those that were able to successfully splice out the old sequence as well as incorporate the new sequence, all were shown to be genetic mosaics; that is, some of the cells in the embryo had been successfully treated and others had not. They also found evidence of a substantial amount of off-target activity in these cells, with a large number of (presumably) cas9-induced indels through exome sequencing[16].

As Huang's study shows, germ-line CRISPR/Cas9 treatments are far from ready for the clinic, unlike comparable treatments targeting somatic cells. These germ-line treatments carry a number of other issues as well as the technical challenges that Huang describes—their potential long-term health effects are completely unknown, and their clinical utility is questionable since genetic screening permits the selection of healthy embryos in any case. Developing the ability to genetically modify embryos could also potentially open the door to treatments with no explicit medical purposes, but rather intended to insert culturally desirable traits—that is, eugenics. As such, the genetic modification of germ-line cells is a highly controversial topic and its future in the clinic is far from a certainty.

As the technology continues to develop, it seems clear that it will find new applications in continually broadening settings. Currently, a major limiting factor for when and where CRISPR/Cas treatments can be used is limited essentially by the

[16] Puping Liang, Junjiu Huang et al. "CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes." Protein and Cell, May 2015

delivery of the complex. It is necessary to actually physically remove the cells that you want to treat to be sure that you don't accidentally target the wrong cells altogether, and so that you can screen the cells for off-target effects before you reimplant them. Naturally, this greatly limits the cell types you can genetically modify.

However, this may not necessarily be the case in the future. Once CRISPR/Cas treatments have become better established, it's possible that they will have such high fidelity and efficiency that it might not be necessary to screen cells and reimplant them to ensure the safety of the patient. It could eventually be possible to actually deliver the treatment directly into the body, perhaps using a viral vector that will only bind specifically to one type of cell before injecting the gene editing construct, which would help to ensure that only the desired tissues are affected. Somebody with Duchenne muscular dystrophy could then simply inject or inhale an aerosol of virus which could travel to every muscle cell in their body and replace the dysfunctional gene with a normal copy.

As the technology becomes safer, it will also be possible to target genes that are not immediately life-threatening but may have some clinical benefit nonetheless. For example, people with certain permutations of two genes, BRCA1 and BRCA2, are significantly more vulnerable to developing breast cancer sometime in the future. If CRISPR/Cas9 becomes refined enough that it can be used in situations where the risk of off-target mutations would be less than the benefit of inserting another subtype of BRCA into cells in relevant tissues—or indeed, replacing any number of genes which may contribute to disease phenotypes—it could prove to be a significant boon to public

health. Medicine could become a highly personalized and preventative field where certain disease causing genes are identified and eliminated early in life in much the same way that people are currently vaccinated against transmissible disease. This will of course be limited by the safety of the treatments, which will likely never be perfect, and by our ability to access relevant tissues.

Although the technology is still in many respects in its infancy, CRISPR/Cas9 is already showing strong promise in terms of its potential clinical applications. As new Cas9 variants are discovered with improved fidelity and efficiency and as new potential gene targets are discovered, these applications will only become more numerous. The labs and hospitals testing new CRISPR/Cas9 based treatments will similarly increase in number. Although this could prove to be a positive development in many respects, the low technical barrier that the use of CRISPR/Cas9 poses is something of a double edged sword. On the one hand, more labs working with the technique means that more treatments will be developed for a wider variety of illnesses. On the other, it has to be acknowledged that some of the labs using CRISPR/Cas9 will exist in regulatory environments that are less stringent than others. The dangers that a poorly designed gene editing treatment could pose to patients are significant.

Chapter II – The Evolution of Scientific Regulation

In the late 1960s and early 70s, the discovery and isolation of restriction endonucleases (viral enzymes which create double stranded breaks in DNA strands) allowed scientists for the first time to combine together DNA from multiple sources[17]. They could insert a target gene into a bacterial plasmid along with a selectable marker like ampicillin resistance, and induce a group of bacteria to absorb and replicate the plasmid. This product could be purified and used in future experiments. The scientific implications of this technology and its successors have been enormous—recombinant DNA is used to produce medicines including insulin and factor VIII, and to create genetically modified organisms for use in medicine, agriculture, and other industries.

Although its manifold potential uses made it a very attractive subject for researchers, recombinant DNA was seen as a technology with far-reaching and if misused, potentially devastating effects. Scientists familiar with the technology were worried that viruses carrying recombinant DNA could potentially introduce new genes into humans if proper safety measures were not taken. Equally concerning was the rising public concern about recombinant DNA, which was reaching a fever pitch[18].

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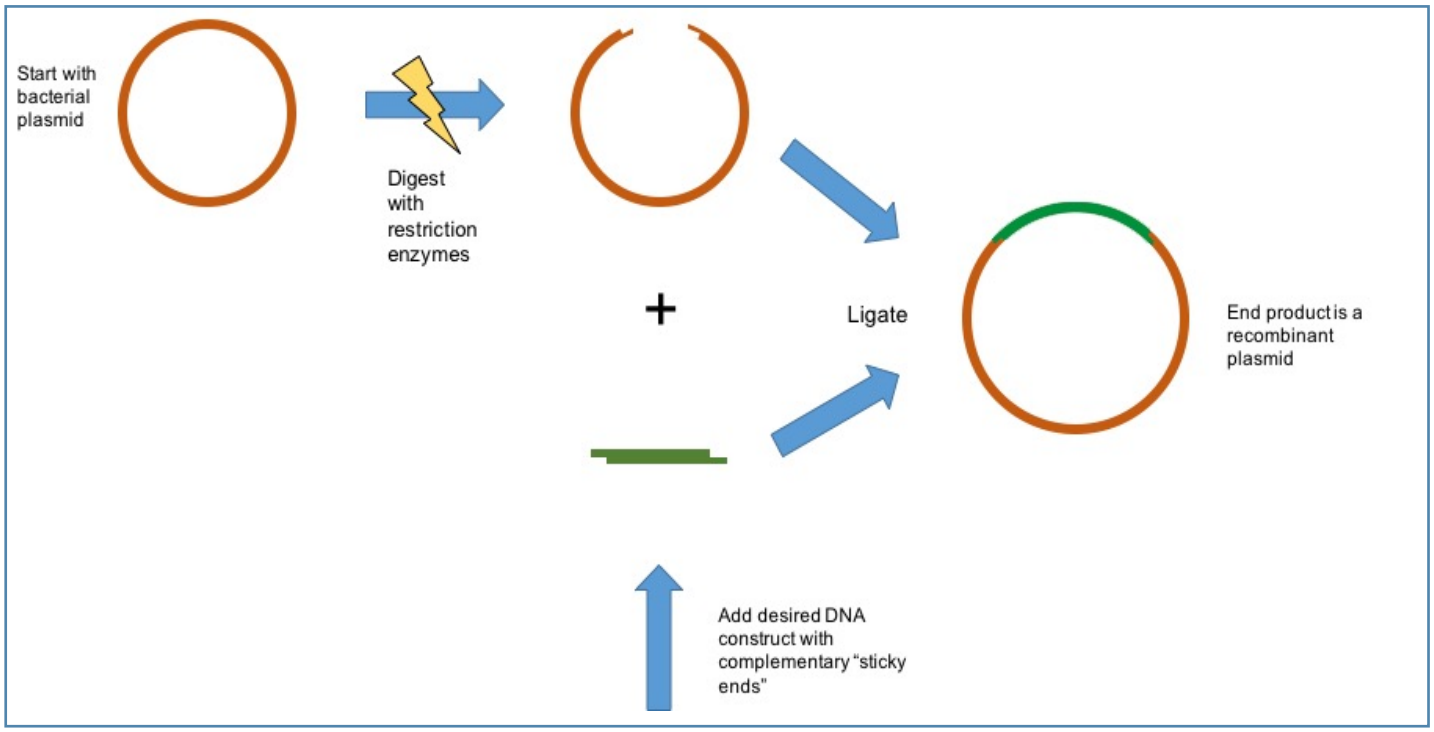
[17] Jackson, D.; Symons, R.; Berg, P. "Biochemical method for inserting new genetic information into DNA of Simian Virus 40: Circular SV40 DNA molecules containing lambda phage genes and the galactose operon of Escherichia coli". *Proceedings of the National Academy of Sciences of the United States of America*. 1972.

[18] Paul Berg, MF Singer. “The recombinant DNA controversy: Twenty years later.” Proceedings of the National Academy of Science, September 1995.

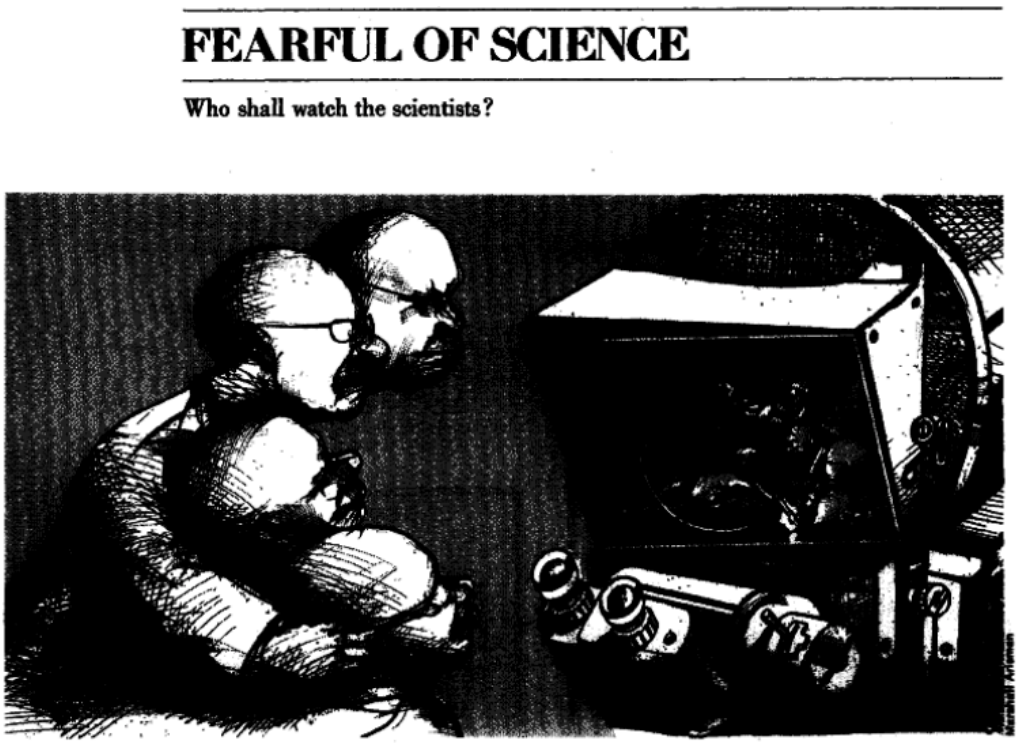


A target gene and plasmid are both digested with matching restriction enzymes to produce "sticky ends." They are then enzymatically ligated together. The recombinant plasmid can be amplified by growing it in competent bacterial cells, and then purified.

Members of the media and scientists alike speculated that bacteria containing recombinant DNA could be accidentally released into the general population, causing disease or even epidemics[19]. Many recombinant DNA experiments were performed on *E. coli*, a bacteria that commonly lives in the human gut. It is not unheard of for scientists working with these bacteria to become sick after accidental exposure; thus critics reasoned that there was a non-zero chance of recombinant DNA being released into the

[19] Judson, Horace Freeland. "Fearful of Science: Who Shall Watch the Scientists?" Harper's 1975

general population after such an event. They termed this “The Andromeda Scenario,” after the then-popular Michael Crichton novel “The Andromeda Strain,” in which scientists unwittingly unleash an alien microbe that nearly destroys the world[20]. Most thought this scenario unlikely, since scientists exposed to *E. coli* from the lab rarely pass it on to additional people, however the remote possibility was enough to stimulate concern. Still others worried that the development of recombinant DNA technology would lead to attempts to alter the genetic makeup of the human race through eugenics,



FEARFUL OF SCIENCE

Who shall watch the scientists?

A 1975 Harper’s Magazine article by Horace Freeland Judson that discusses concerns in the public and within the scientific community around recombinant DNA.

[20] “Recombinant DNA: Clashing Views Aired.” *Science News*, vol. 111, no. 12, 1977, pp. 181–181., www.jstor.org/stable/3961707.

and wondered what the most ruthless proponents of eugenics such as Adolf Hitler might have done if they had access to comparable technology[21].

Paul Berg, 1980. NIH Library of Medicine.

In 1974, a US National Academy of Science (NAS) committee chaired by an influential Stanford biochemist named Paul recommended a temporary moratorium on any recombinant DNA experiments that could have dangerous consequences (essentially any experiments that would confer antibiotic resistance or potentially cancer-causing mutations)[22]. Berg decided to organize the committee after concerned colleagues pressured him to cease one of his own experiments, which would have inserted potentially pathogenic recombinant DNA into *E. coli*, and was believed to pose a biohazard risk[23]. Although the moratorium was heavily criticized across scientific communities and taken as evidence in the eyes of the press that recombinant DNA technology was indeed the threat that they suspected, it proved to be a successful measure in that it prevented potentially hazardous experiments from being performed before a regulatory framework existed to help with risk containment[24].

The committee also suggested that the NIH form a permanent committee to oversee research using recombinant DNA molecules in the future, to assess risks and

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[21] Ibid
[22] Paul Berg, MF Singer. "The recombinant DNA controversy: Twenty years later." Proceedings of the National Academy of Science, September 1995.
[23] Carmen, Ira H. "Cloning and the Constitution: An Inquiry into Governmental Policymaking and Genetic Experimentation." University of Wisconsin Press, 1985.
[24] Paul Berg, MF Singer. "The recombinant DNA controversy: Twenty years later." Proceedings of the National Academy of Science, September 1995.

determine what kinds of procedures should be taken to alleviate those risks. This committee was later formed as the Recombinant DNA Molecule Program Advisory Committee, today known as the Recombinant DNA Advisory Committee[25]. Despite the opposition, the NAS recommendation for a moratorium was respected around the world over the following months, even though compliance was completely voluntary. Many groups, Paul Berg's included, halted any experiments using recombinant DNA molecules that were likely to confer carcinogenic or antibiotic resistant properties to their hosts[26].

The committee also recommended that an international conference of experts be called to discuss the potential risks and benefits of recombinant DNA technology, as well as possible ways that any risks could be reduced or eliminated. Nine months later, in February of 1975, this recommendation resulted in the Asilomar conference. This conference constituted one of the first major efforts by the international scientific community to self-regulate; it was clear that failing to produce adequate regulation would result in likely more constrictive regulation by outside governmental forces due to the growing fear and ignorance surrounding recombinant DNA technology. Indeed, if the most fearful of the scientists were correct in their risk assessment and there *was* a disaster in which people were harmed by recombinant organisms, the subsequent regulation could be devastating for the field. As such, there was a concerted effort to develop a way to establish an estimate of risk for a given set of experiments, and to

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[25] Paul Berg et al "US Summary Statement of the Asilomar Conference on Recombinant DNA Molecules." National Library of Medicine. NIH, Apr. 1975

[26] Paul Berg, MF Singer. "The recombinant DNA controversy: Twenty years later." Proceedings of the National Academy of Science, September 1995.

create guidelines to allow scientists to manage that risk without undermining their research.

In developing guidelines for the use of recombinant DNA, the conference attempted to adhere to two major principles, "(i) that containment be made an essential consideration in the experimental design and, (ii) that the effectiveness of the containment should match, as closely as possible, the estimated risk[27]." They wanted to ensure essentially that appropriate measures were taken to account for any potential dangers present without making it unduly difficult to perform the experiments necessary to carry the field forward. They acknowledged the limitations of their understanding, which were considerable in the early days of the technology, and agreed that wherever uncertainty was present it was better to err on the side of caution.

The conference decided that cloning experiments using *E. Coli* and many other common bacterial models posed a minimal public health hazard, and could be safely performed in most labs on an open bench. This recommendation extended to vectors which were unlikely to survive outside of the laboratory, which could also be used with minimal containment. In contrast, any recombinant DNA experiments using potentially pathogenic vectors or targeting animals were determined to require a high degree of containment. Similarly, the introduction of genes that could potentially be harmful to humans, as well as genes that come from highly pathogenic organisms or using vectors that are known to infect humans, were to be deferred indefinitely. They noted that scale

[27] Paul Berg et al "US Summary Statement of the Asilomar Conference on Recombinant DNA Molecules." National Library of Medicine. NIH, Apr. 1975

was a matter of importance, that large scale experiments required more caution than smaller ones due to the increased quantity of potentially dangerous material[28].

In the summary of the conference published in the Proceedings of the National Academy of Sciences in 1981, it is clear that the organizers expected a high level of cooperation from scientists and regulatory agencies around the world. They seem confident that their recommendations will be respected, remarking that "through both formal and informal channels of information within and between the nations of the world, the way in which potential biohazards and levels of containment are matched [will] be consistent[29]." They state that although not every country has a regulatory body that has drawn up guidelines for use of recombinant DNA, scientists who find themselves without formal regulation should simply use the summary of the proceedings as a guideline.

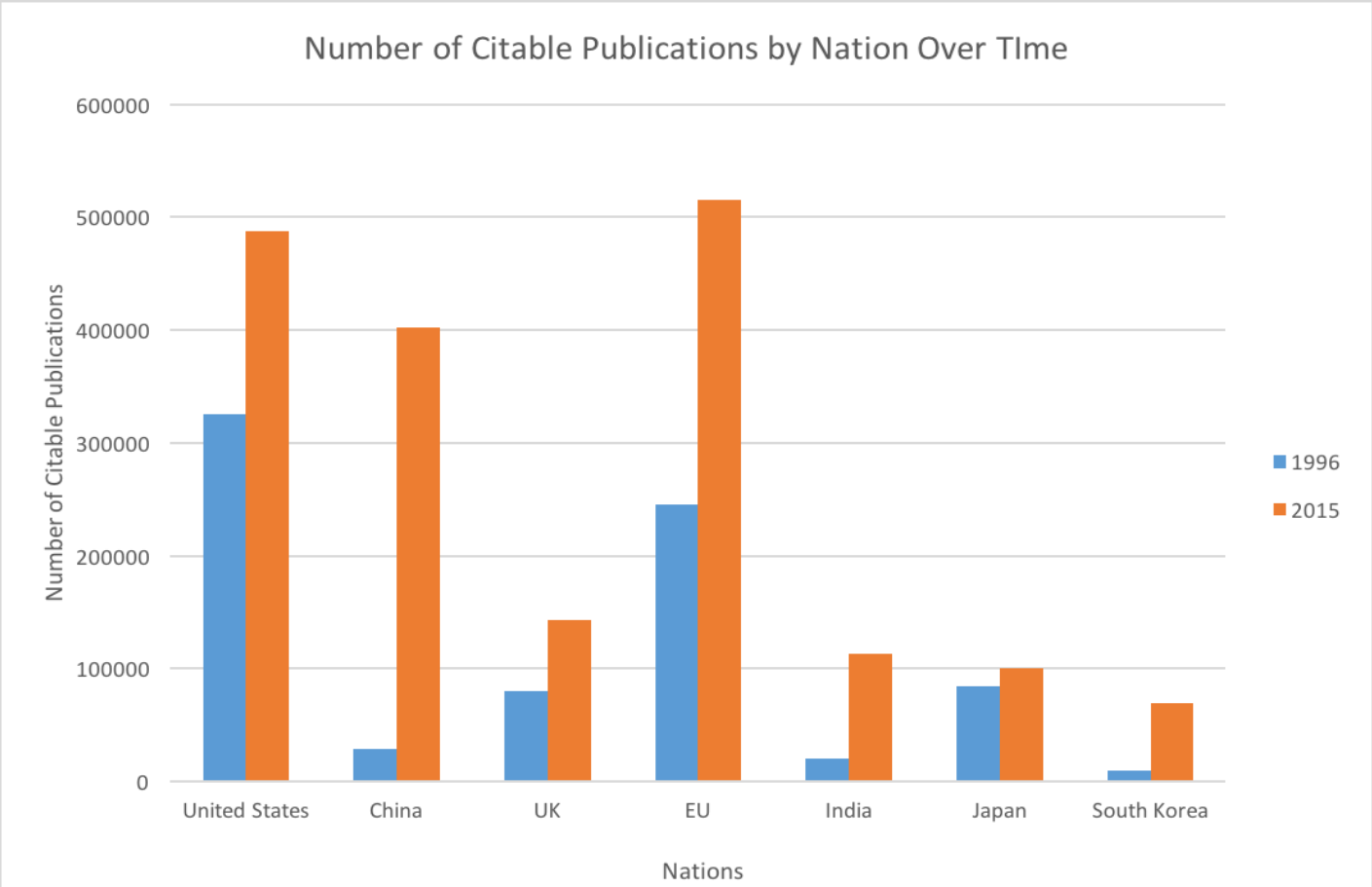
For its time period, this may well have been a reasonable expectation—in the 70s and 80s, biological and biomedical research was restricted almost entirely to North America and Europe. With such a small cohort of mostly westernized nations, there were a relatively small number of cultural, legal, and political differences to account for in shaping and implementing these guidelines; the scientific community was small and centralized enough that an agreement reached by its leaders could reliably achieve universal recognition.

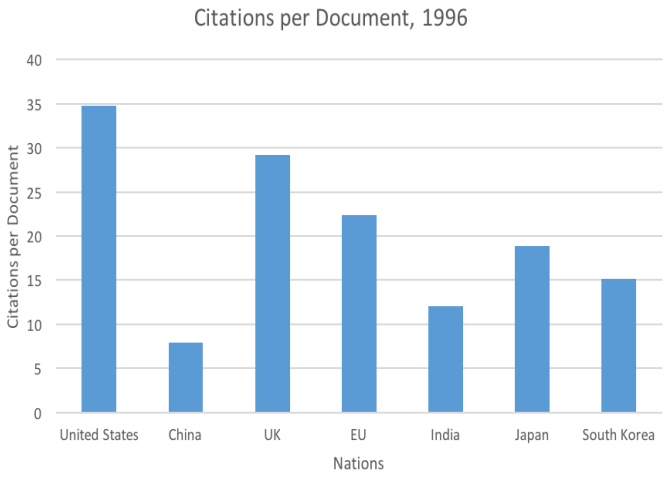
Indeed, the conference was widely considered to be an outstanding success, and its conclusions and recommendations with regard to safety were globally respected for

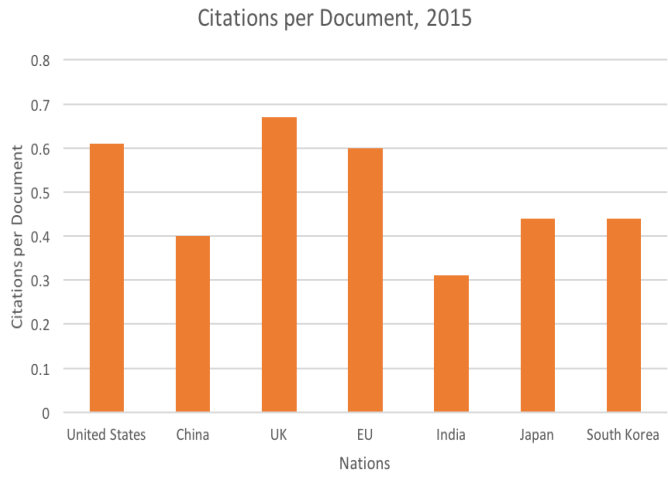
[28] Ibid
[29] Ibid

years. As Paul Berg and Maxine Singer noted in a retrospective document published 20 years after the Asilomar Conference, “Literally millions of experiments, many even inconceivable in 1975, have been carried out in the last 20 years without incident. No documented hazard to public health has been attributable to the applications of recombinant DNA technology[30].” In fact, the impact of the conference went far beyond its initial scope and goals (which it clearly achieved beyond all expectation). It set an important precedent for dealing with new, potentially dangerous technologies—it was proof that leaders in the field could assemble, discuss the potential risks and benefits of the new technology, and make recommendations to formal regulatory agencies and to their colleagues around the world. As Berg acknowledges, their guidelines were initially stricter than necessary in order to safeguard against their limited understanding of recombinant organisms. However, the rules left plenty of room for discovery to continue, and were slowly relaxed as more and more was learned. The Asilomar conference showed that scientists were capable of regulating themselves and each other, and helped to reassure the public that such was the case. This capacity for self-regulation ensures that science can continue safely without regulation that unduly suppresses innovation.

[30] Paul Berg, MF Singer. “The recombinant DNA controversy: Twenty years later.” Proceedings of the National Academy of Science, September 1995.







There has been remarkable growth scientific output from China and India in particular over the past two decades. Although number of scientific publications is not necessarily the best measurement for productivity—in particular, it does not measure the influence or quality of these publications—it can at least provide some idea of the increase in scientific investment that has taken place in these nations. Citations per document does provide a rough metric for average publication quality (though this number has dropped universally over time as overall number of

In contrast to the relatively small communities of the 70s and 80s, contemporary scientific communities are no longer localized to a handful of nations. In particular[31], China and India have developed into extremely influential and productive science hubs. To a lesser extent, countries like Brazil, Spain, South Korea, Iran, Turkey, and Malaysia have all dramatically increased their scientific output between the Asilomar conference and the present day in terms of total papers published. There is still a fairly substantial gap in terms of quality of publication output—although the raw number of papers from China currently rivals that of the EU, the number of papers published in high impact journals or receiving numerous citations has lagged behind[32]. Even so, the simple increase in raw publication numbers is indicative of increased scientific investment and participation from a wider variety of nations and culture, even if the quality of these publications on average cannot yet be said to rival that of more traditional centers for scientific research.

This decentralization poses a formidable challenge to any formal or informal regulatory bodies seeking global cooperation on issues of research safety and ethics. Without entering into a discussion of moral relativism, it seems clear enough to say that it is more difficult to agree upon regulatory guidelines when ethical values are not necessarily conserved across cultures. An acceptable risk to the public in one culture may not be considered acceptable in another. As such, agreeing on any significant international framework for regulation can be extremely difficult.

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[31] Scopus Scientific Abstract and Citation Database. Elsevier, 2017.
[32] Ibid

Chapter III - Regulating CRISPR/Cas9

Indeed, more recent international regulatory efforts comparable in size and scope to the Asilomar have faced substantially greater challenges than their predecessors. CRISPR/Cas9 particularly has presented a slew of potential legal and ethical hazards to scientists and regulators. The relatively uncontroversial modification of non-germ line cells using the technique is delicate enough—a major clinical misstep at this stage could provoke regulatory hurdles that would set the technology back years. However, in May of 2015 a team of Chinese scientists led by Junjiu Huang at Sun Yat-sen University published a study in *Protein & Cell* (after supposedly being rejected from higher profile journals due to ethics objections) describing their efforts to use CRISPR/Cas9 to introduce a gene into human embryos[33]. Genetic modification of germ-line cells is explicitly illegal in many Western European nations; although it is not so in the US, the National Institute of Health's Recombinant DNA Advisory Committee has announced that it will not approve any experiments performing such modifications[34]. As such, these were experiments that would have been impossible in the US that many found to be ethically outrageous[35].

Ironically, the announcement of germ-line modifications with CRISPR/cas9 came only shortly after a highly publicized commentary published in *Nature* by a group of leading US stem cell and regenerative medicine researchers on that very topic. Their commentary called for a voluntary moratorium on any research attempting to genetically

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[33] David Cyranoski, Sarah Reardon. "Chinese scientists genetically modify human embryos." Nature News, April 2015.
[34] Edward Lanphier et al. "Don't edit the human germ line." Nature News, May 2015.
[35] Cyranoski et al. "Chinese scientists genetically modify human embryos." 2015.

modify human germ cell lines[36]. They note that the availability of tools for genetic manipulation has exploded, and that it is clear that modifying embryos using zinc finger nucleases or CRISPR/Cas9 is possible. However, they point out from a technical perspective that the modification of germline cells is totally different from that of somatic cells. Because germline cells will eventually develop into every type of tissue in the body, it is much harder to predict what kinds of effects a given genetic manipulation will have on the health of the patient even if that manipulation is performed perfectly, without any unintended genetic alterations. As we know, CRISPR/Cas9 *does* bind at off-target sites and induce random mutations. The effects of any such mutations in germline cells would likely be amplified tremendously compared to their effects in somatic cell treatments. As such, the patient safety issues involved in attempts at genetic modification of germline cells are impossible to justify.

The team also raise a number of ethical concerns with germ-line genetic experiments, noting that that "many oppose germline modification on the grounds that permitting even unambiguously therapeutic interventions could start us down a path towards non-therapeutic genetic enhancement. We share these concerns[37]."In fact, the group argues that there is no real therapeutic justification for attempting to modify germline cells in the first place. In circumstances where CRISPR/Cas would be an option, i.e. under conditions where IVF is being employed, it is already possible to perform genetic screening of embryos for disease and to simply select the healthy embryos and discard the rest. Attempting to develop techniques to safely modify germline cells is

[36] Lanphier et al. "Don't edit the human germ line." 2015
[37] Ibid

therefore unnecessary, they say, and clearly opens a door for those who would abuse the technique to make medically unnecessary genetic modifications.

Finally, Lanphier and his colleagues state the need for international discussion and consensus. They acknowledge that germ line research is already unlikely to occur in the west and seem to hope that they can achieve at least temporary agreement from their counterparts in other parts of the world based on their concerns about the health risks of germline modification. If germ-line experiments are permitted to take place, they fear that the public will not appreciate the difference between germ-line editing and somatic cell editing and that there will be substantial backlash against genome editing as a whole.

Huang has argued that his study demonstrates how different human embryonic cells are to other models like mouse embryos and human somatic cells. He says his results show that CRISPR/Cas9 is far from ready for use in humans, noting that "If you want to do it in normal embryos, you need to be close to 100% [efficiency][38]." However, his group implicitly supports continued efforts in this line of study, arguing that "further investigation of the molecular mechanisms of CRISPR/Cas9-mediated gene editing in human model is sorely needed[…]before any clinical application[39]." This statement lends validity to fears that Huang's study will prompt other groups to attempt to improve on his techniques.

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[38] Cyranoski et al. "Chinese scientists genetically modify human embryos." 2015.
[39] Liang et al. "CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes." 2015

When Paul Berg called for a voluntary moratorium in the early days of recombinant DNA, the moratorium was accepted until such rules were developed during the Asilomar conference. Under similar circumstances in 2015, the moratorium established by Lanphier et al was broken almost immediately. Clearly then, the progressive decentralization of scientific research over the past several decades has had a very real effect on how standards of practice can be established. In the 70s, it was reasonable to expect cooperation from the majority of researchers if a technique was believed to pose a potential hazard to public health. In the present day, formal agreements developed by international bodies are necessary to establish clear universal rules.

Although this study clearly took place well before the publication of Lanphier's commentary—in fact, the commentary was written explicitly as a response to rumors that such studies had been performed—an article published in *Nature News* shortly after Huang's study notes that "at least four groups in China are pursuing gene editing in human embryos[40]." Clearly then, the recommendations of a panel of senior scientists based in a single nation has far less impact on the behavior of the scientific community as a whole than it did in the 70s.

In December of 2015, the National Academy of Science, the Chinese Academy of Science, and the Royal Academy (UK) held an international joint summit to discuss gene-editing, including research using CRISPR/Cas9. They hoped to help develop a dialogue between nations with starkly different regulatory environments, to "establish norms

[40] Cyranoski et al. "Chinese scientists genetically modify human embryos." 2015.

concerning acceptable uses of human germline editing and to harmonize regulations, in order to discourage unacceptable activities while advancing human health and welfare[41]." The agenda included research targeting somatic cell lines, which the organizers (including Paul Berg) acknowledged as potentially risky but as able to be "appropriately and rigorously evaluated within existing and evolving regulatory frameworks for gene therapy[…]regulators can weigh risks and potential benefits in approving clinical trials and therapies[42]." Germ-line editing also formed a significant portion of the agenda, and was framed in a much more critical light.

The organizers note in their summary of the conference that modifying germ-line cells in a clinically useful way is extremely difficult due to many of the same technical issues raised by Lanphier et al, and subsequently confirmed by Liang et al. They also raise concerns about the irreversible nature of genetic changes to such cells—since germ-line cells will develop into reproductive cells, any changes (therapeutic or otherwise) will be inherited by future generations. If new genes are introduced into such cells, it could permanently change the genetic makeup of the human race with impossible to predict consequences. If protocols *are* developed for germ-line editing, even if they are intended therapeutically, they could potentially be adapted by unscrupulous experimenters to provide non-therapeutic genetic enhancements. Since IVF is an expensive process, the availability of these enhancement treatments could worsen pre-existing social inequalities. The conference ultimately concluded that no proposed therapies have

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[41] NIH, CAS, NAS, TRS. "On Human Gene Editing: International Summit Statement." International Summit on Human Gene Editing: A Global Discussion. December 2015
[42] Ibid

demonstrated sufficient justification based on a cost/risk analysis, and that no acceptable legal framework currently existed to regulate such therapies. As such, they recommended that germ-line genome editing experiments be discouraged until the topic is next revisited.

As of today, the international scientific regulatory cooperation that the organizers of the CRISPR conference described has not materialized. Chinese scientists in particular continue to perform experiments using CRISPR/Cas9 in human germ-line cells. In April of 2016, a group at the Guangzhou Medical University under Yong Fan published a report in the *Journal of Assisted Reproductive Genetics* in which they claimed to have unsuccessfully attempted to confer HIV immunity to human fetuses by inserting an allele called CCR5delta32 using CRISPR. They ran into many of the same issues as their predecessors—and included the same assurances that they knew the technology was not ready for the clinic—however it is clear that the development of germ-line treatments with CRISPR/cas9 was not halted by the recommendations of the summit on gene editing[43].

Faced with a lack of international cooperation, the National Academy of Science has made a reversal on their germ-line editing policy. Although they still acknowledge the dangers posed by germ-line gene editing discussed at their 2015 summit, they announced in February of 2017 that, pending further review, they will suspend their ban on such research sometime in the near future. In cases where there are no other therapeutic options—where the only solution would be to discard the afflicted embryos

[43] Callaway, Ewen. “Second Chinese team reports gene editing in human embryos.” April 2016.

altogether—they will consider accepting research studies that would attempt to repair these cells at the genetic level. Under these criteria, germ-line therapies could potentially be used for lethal genetic disorders like Huntingtons and Tay Sachs[44]. The clinical utility of such therapies is questionable, however.

People who know that they are potential carriers of such diseases often choose to have in vitro fertilization, which is of course the scenario where germ-line editing would most likely be usable. The current protocol for such cases is to genetically screen each of the embryos and select those that do not carry the genetic disorder before freezing or discarding the rest. This method is simple, relatively inexpensive, and very effective. As discussed previously, germ-line editing is none of those things. Even if it is developed over time to the point where it can be performed with minimal risks—and this is hard to imagine, since the effects of germline editing will likely vary across treatments and could take decades or even generations to manifest themselves—it is not clear that it would have any clinical utility. It does not offer any advantages over the genetic screening of embryos and would seemingly always carry at least some nominal risk. It could potentially allow embryos that would otherwise have been discarded to be implanted, but since IVF involves the fertilization of many more embryos than are needed (this helps to ensure that at least a few will be viable), this too would have limited utility—if one embryos is not discarded, another will be.

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[44] National Academy of Science. "With Stringent Oversight, Heritable Germline Editing Clinical Trials Could One Day Be Permitted for Serious Conditions; Non-Heritable Clinical Trials Should Be Limited to Treating or Preventing Disease or Disability at This Time." February 2017.

Although the implications of this report are potentially disturbing, it seems to be based in a realistic assessment of the current status of the technology. As Alta Charo, the chair of the NAS Committee on Human Gene Editing states, "If we have an absolute prohibition in the United States with this technology advancing, it is not like it will not happen[45]." These kinds of studies are moving forward elsewhere in the world, and will likely continue to do so. At least if we have them done in the United States in a regulatory environment that we can actually control, we can make sure that all relevant materials are obtained ethically, that patient safety is ensured to the highest possible standards, and that the results are properly scrutinized for replicability. These are ideas that have been driven home over the past decade by the regulatory environment surrounding stem cell research, which has been strict enough that many important and necessary studies using stem cells cannot easily be performed in the west. Instead, they are undertaken in regions of the world with less strict regulatory environments and often with less developed scientific infrastructure.

In the west, embryonic stem cell research has been a highly political issue surrounded by volatility for several decades. Major research centers including Germany and France have long-standing bans on the creation of embryonic stem cell lines, though in some cases these lines can legally be imported from other countries with less strict regulation, such as the UK[46]. Since 2001, the US has taken a conservative stance towards stem cell research, banning the appropriation of federal funding for research creating

[45] Harmon, Amy. "Human Gene Editing Receives Science Panel's Support." New York Times, February 2017.
[46] Dhar, Deepali, and John Hsi-en Ho. "Stem Cell Research Policies around the World." The Yale Journal of Biology and Medicine, 2009.

human stem cell lines without banning the creation of these lines themselves. This resulted for many years in the stagnation of stem cell research in these more restrictive nations, even during periods when some amount of funding was available; the lack of stability of this funding made a potentially lengthy research project an uninviting prospect for many. This has improved somewhat over time with the creation of several state and private funds specifically dedicated to stem cell research[47].

In contrast, non-western research centers like China, India, and Japan have been unhampered by the hang-ups affecting the US and parts of Europe, which often seem to be based at least partially in the religious belief that life begins at conception. In China in particular, stem cell research has flourished. A group led by Fiona Murray published findings in the New England Journal of Medicine in 2006, stating that "China is in fact accumulating substantial expertise in this area[…and that] China could become a major force in the stem-cell industry," while acknowledging simultaneously that a lack of government funding and a low average wage remained a major roadblock to the development of a significant stem cell biotechnology industry in the country[48]. Murray also notes that because of the lack of a single centralized regulatory body comparable to the FDA, it is easier for a medical researcher to engage in small scale clinical trials in China, which could potentially provide them with an edge against competitors. Indeed, a 2013 study by Jingyuan Luo published in *PLOS One* found that between 2000 and 2010 they number of Chinese authored stem cell papers published yearly had increased

[47] "Obama overturns Bush policy on stem cells." CNN Politics, March 2009.
[48] Fiona Murray et al. "Bit Player or Powerhouse? China and Stem Cell Research." New England Journal of Medicine, September 2006.

tenfold. Furthermore, while the quantity of stem-cell publications in the west stagnated or grew modestly over the same time period, the number of papers involving international collaborations nearly doubled, from 20.9% to 36%[49]. In short, the absence of participation from scientists in the west has helped stem cell research to flourish elsewhere in the world. Although many of these newer scientific centers have produced a large volume of high quality research, there have also been significant missteps.

Hwang Woo-suk, a disgraced South Korean stem cell researcher, caused significant turmoil in his field when it was discovered amongst a litany of other ethical violations that his claims of having cloned embryonic human stem cells were fraudulent. His questionable practices first came to light in May of 2004, when a *Nature* article by David Cyranoski brought forward claims that Woo-suk had unethically and possibly illegally obtained human eggs for his stem cell cloning experiments[50]. It discussed the possibility that he had gone so far as to coerce one of his students, Ja Min Koo, into donating her eggs for the project. Even if the donation was purely voluntary, Cyranoski points out that the use of her egg cells was probably still unethical:

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[49] Jingyuan Luo, Kirstin R. W. Matthews. "Globalization of Stem Cell Science: An Examination of Current and Past Collaborative Research Networks." *PLOS One*, September 2013.

[50] David Cyranoski. "Korea's stem-cell stars dogged by suspicion of ethical breach." *Nature News*, May 2004

> "The information posted with the paper states: "Neither donors nor their family, relatives or associates may benefit from this research." Koo, who was a co-author on the paper, arguably did stand to gain professionally from its publication[51]."

Woo-suk eventually admitted that he had unethically sourced the eggs used in his studies, and was later indicted on counts of fraud and embezzlement after it became clear that he had falsified the data presented in the study altogether, and had stolen or misspent $2.6 million from federal and private donations which he initially claimed to have spent on his research[52]. As a result, he was fired from his faculty position in Seoul and resigned from many of his other official positions. He was ultimately convicted for all but the fraud offenses, receiving a 2-year prison sentence[53].

The former "Pride of Korea" cost the South Korean ministry of science and technology millions of dollars with his falsified studies. His status within the country prior to his indictment meant that he was nearly above reproach – when allegations first appeared, "Many commentators said it was unpatriotic to challenge someone who had given the country a lead in such a promising new area[54]." As such, independent bioethicists based in Seoul who led investigations alongside *Nature* into Woo-suk's conduct and the veracity of the published results initially faced substantial resistance

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[51] Ibid
[52] The Associated Press. "Disgraced Korean Cloning Scientist Indicted." *New York Times*, May 2006.

[53] David Cyranoski. "Woo Suk Convicted, but not of fraud." *Nature News,* October 2009.
[54] "South Korea Stem Cell Success 'Faked.'" *BBC News*. December 2005.

from his supporters[55]. Scientific fraud occurs all over the world—certainly the west has had its share of controversies and fraudulent studies—but it thrives in conditions where the power of an individual or institution are allowed to complicate the process of peer review, and where criticisms of irreproducibility are dismissed due to the prestige of the lab that produced the original results.

It should be noted that in comparison to other major centers of stem cell research, South Korea has a relatively robust regulatory system in place. That Woo-suk's corruption was discovered, exposed, and prosecuted even despite his prestigious status within the South Korean scientific community is testament to the quality of South Korean federal regulators and the national scientific regulatory environment generally. In regulatory environments in which fraud and unethical practices are systemic and widespread, it is unlikely that the discovery of such practices would produce the level of controversy that Woo-suk generated. This is more closely characteristic of the culture in some scientific fields in China than South Korea; the Chinese Food and Drug Administration estimated in 2016 that some 80% of 1,622 clinical trials performed in China contained data that was likely fabricated, or that otherwise failed to meet government standards[56]. If accurate, this report indicates a pervasive culture of scientific fraud that casts serious doubt on stem cell studies performed in Chinese universities.

Because these stem cell studies were undertaken in regions which had less well-established and rigorous regulatory environment, in many cases the studies were carried

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[55] David Cyranoski. "Korea's stem-cell stars dogged by suspicion of ethical breach." 2004
[56] Fiona Macdonald. "80% of Data in Chinese Clinical Trials have been Fabricated." Science Alert, October 2016.

out less ethically than they might have been in the west had regulations been more permissive. The published results may also be less reliable, which obviously poses huge problems to efforts to move the research forward in a clinical setting. Testing their reproducibility also becomes more difficult, since repeats of the basic studies might not be able to receive approval in the west. As such, in developing regulations for future technologies—particularly germ-line genome editing using CRISPR/cas9—it is important that guidelines do not result in complete prohibition. Keeping even-handed guidelines will help to ensure that studies will be performed using ethical and methodological standards that would be considered acceptable by the scientific community at large.

Even so, the distinction that NAS Committee on Human Gene Editing chair Alta Charo draws between justified and unjustified germ-line research with her statement that "we are not talking about designer babies, we are talking about healthy babies" is flawed to a certain extent[57]. She argues that clinical trials in germ line cells could be permitted if the science progresses to a point where it can be performed safely with a high degree of confidence, and "if we develop a regulatory system capable of making sure it is used only for those purposes, and not for anything unwarranted or untoward[58]." The question remains whether such a regulatory system is truly possible.

Efforts to modify non-germline cells with CRISPR are straightforward, relatively speaking, and have been widely undertaken. The safety issues that they pose are similar

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[57] National Academy of Science. "With Stringent Oversight, Heritable Germline Editing Clinical Trials Could One Day Be Permitted for Serious Conditions; Non-Heritable Clinical Trials Should Be Limited to Treating or Preventing Disease or Disability at This Time." February 2017.
[58] Ibid

to those posed by earlier gene editing techniques, and as such the regulatory framework necessary to monitor such treatments is already well-established. In contrast, no gene editing technology has yet been able to perform the successful genetic modification of germline cells. Obviously, no regulatory framework has yet been established to deal with this eventuality, and as such it is not clear how such a framework could be implemented effectively. Once the technology for making genetic modifications for human embryos exists, it will not be a trivial matter to ensure that it is used exclusively for well-tested clinical purposes. The NIH may hold researchers in the US to a high standard to prove that their treatments are safe and that they have a valid medical justification, but they have no way to do the same to research that occurs in other nations. If even a single country with a moderately sized biomedical infrastructure fails to fully regulate germ-line modification, techniques developed in the US could potentially be adapted to ends that we would not consider ethically acceptable. The only way it would be even remotely possible to ensure that gene-editing technology developed for the clinic is not misused would be to develop an international agreement on what kinds of germ-line modification research can and cannot be conducted. Although such an agreement could prove to be hugely beneficial, efforts at attaining international agreement on regulatory standards in the past have encountered serious roadblocks.

In November of 2015, representatives from 195 nations met for in Paris for the United Nations Climate Change Conference to discuss a potential international accord to reduce greenhouse gas emissions and thereby to combat climate change. Climate change is a global phenomenon that every nation contributes to. If it is allowed to continue

unabated, every nation also stands to face a series of economic and humanitarian crises of an immense magnitude. As such, there is both a considerable need and a strong incentive for international cooperation on taking steps to abate as much damage from climate change as is reasonably possible[59]. In apparent recognition of this fact, 194 of the nations in attendance signed the accord and 141 have since ratified it—far more than the 55 parties accounting for a total of 55% of global greenhouse gas emitters required for the accord to go into effect.

Implementation of the accord, however, has been weak to non-existent. This is in part due to the vague terms of the agreement—it does not specify a target emissions goals for different groups of nations, but rather permits all of them to set their own goals. It merely states that the overall aim of the agreement is to "keep a global temperature rise this century well below 2 degrees Celsius above pre-industrial levels and to pursue efforts to limit the temperature increase even further to 1.5 degrees Celsius[60]." It has no provisions for punishing nations who fail to meet their own goals or who fail to pursue sufficiently ambitions reductions, or even to define what level of reduction would be considered sufficiently ambitious.

This is problematic, since any nation that develops stricter standards for emissions and pursues more ambitious goals will fundamentally be at an economic disadvantage to nations that do not—green technology isn't cheap, after all, and implementing it causes

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[59] Sutter, John D.; Berlinger, Joshua (12 December 2015). "Final draft of climate deal formally accepted in Paris". *CNN*. Cable News Network, Turner Broadcasting System, Inc. Retrieved 12 December 2015

[60] United Nations Framework Convention on Climate Change. "Paris Agreement: Essential Elements." November 2016.

products from the relevant region to be more expensive and therefore less competitive in the international market. The climate change agreement also calls for developed nations to raise $100 billion annually as incentive to help developing nations reduce their greenhouse gas emissions, partly to offset the damage that this reduction in competitiveness causes, but again it provides no specific framework for who should contribute this money or how it should be raised.

According to the Potsdam Institute for Climate Impact Research, if every nation follows its proposed target emissions reductions global average temperatures will rise by 2.7° C this century, significantly higher than the conferences 2 C° goal and nowhere near the stated "stretch" target of 1.5° C[61]. In fact, very few nations are on track to meet the targets that they have stated—if actual existing policies are continued, the expected warming will reach 3.6° C. Clearly then, despite the accord's ambitious goal and an inherent global incentive to reduce climate change, signatory nations are falling far short of their target.

How can we adapt the lessons learned from the Paris climate agreement to apply to the regulation of genome editing technologies? Clearly for any international accord to be successful, regulators will have to agree on specific provisions for what kind of research can and cannot be performed. The vague targets of the climate agreement were open to interpretation and resulted in vastly different regulatory behaviors within different nations and cultures. As such, a gene editing agreement would ideally state

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[61] Kinver, Mark. "COP21: What does the Paris climate agreement mean for me?" BBC News, December 2015.

explicitly that studies investigating how to edit the genome of germ-line cells will not be permitted; such studies have no clear clinical application, after all. The agreement would also need to set up a board responsible for ensuring that the terms are adhered to by the signatory parties, which in turn would require that signatories provide funding proportionate to their scientific investment to allow that board to function.

The odds of such an agreement being reached are not good, for many of the same reasons that an equivalent agreement was not reached for climate change. Even though the human race as a whole would likely benefit from global cooperation on this issue, any nation that signed an agreement banning certain scientific practices would be at an economic disadvantage to any nation that did not. This prisoner's dilemma-like scenario is difficult to resolve. Furthermore, although there seemed to be a tentative consensus among the nations leading the international gene editing conference in 2015 that germ-line editing was not a desirable aim for the near future, studies in China have continued regardless[62]. This could indicate a difference of values between the west and east—while the US and EU may feel that the potential hazard to public health and risk of eugenics outweighs the potential economic benefits, the same may not be true elsewhere. As such, there may not even be the mutual desire to establish a shared set of regulations that a functional international accord would require. Obviously there is no way for us to compel other nations to adopt our moral standards, so if this is true then no effective international regulatory agreement can be established.

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[62] NIH, CAS, NAS, TRS. "On Human Gene Editing: International Summit Statement." International Summit on Human Gene Editing: A Global Discussion. December 2015

Even if official consensus on regulatory matters can be obtained, enforcement of that agreement may simply not be feasible in some regions. Particularly as certain technologies become cheaper and easier to perform and the barrier to entry is lowered, medical researchers in developing countries may find the prospective financial reward of these experimental procedures, coupled with the lack of regulatory enforcement, irresistible. As such, unless there exists the will to dedicate vast resources to international enforcement, even the best international regulatory agreement possible could prove to be ineffective.

Conclusions and Recommendations

It seems unlikely that an effective and sound internationally standardized set of regulations for germ-line editing can be established despite the apparent hazards that the technology poses. Scientists in the west should focus on developing somatic cell treatments using CRISPR/Cas and on encouraging their counterparts in other research centers to do the same. International conferences like the 2015 international summit on gene editing should be utilized on a regular basis to help homogenize ethical standards amongst leaders in the field all over the world. Even if it isn't possible to obtain formal regulatory agreement from major governing bodies, if a general consensus exists amongst scientists that experiments with germ line cells should be deferred then even those who are unconcerned with the social impact of their work may adhere to this consensus out of fear for their reputation in the eyes of their peers.

Regulatory policy in the US should account for the likelihood that different regulations will probably exist in different places. This means that we need to think about the possibility that germ-line genome editing treatments genuinely designed for clinical use could pave the way for unsafe or clinically unnecessary treatments. In other words, when considering a risk/benefit analysis for a proposed study, we need to account for the potential misapplication of the knowledge that study might produce in regions without strict scientific regulation. Taken with the razor-thin margin by which germ-line gene editing could be justified even without this consideration, it is hard to imagine a case in which it would be acceptable to approve research in this particular

field. As such, the NAS should reverse their current position (again) and refuse to approve any studies using CRISPR/Cas9 in the human germ line.

We should also consider the likelihood that inconsistent regulation between nations could result in the development of 'grey markets' for both research resources (like the human egg cells from Woo-suk's discredited study) and for medical procedures, in which members of one region where a procedure is illegal or cost-prohibitive can travel to another region (usually, but not necessarily, one with less medical regulatory infrastructure) where it is more easily accessible. This could profoundly undermine regulatory efforts—not only do grey markets render them ineffective at controlling access to the procedure in question, they may also actively encourage people to travel to places where a lack of regulatory oversight might result in unsafe medical treatment with potentially unethically sourced medical materials. The social outcome of such regulations can be considered in many regards worse than if more lenient rules had been established. Although banning germ-line research in the west outright might slow the development of these medical grey markets, research will slowly continue elsewhere. If germ-line editing eventually becomes a scientific reality, we will have to reconsider our prohibition against it in order to ensure that it can at least be performed in a relatively safe and ethical environment.

It may not be possible to prevent the development of germ-line genome editing technology indefinitely, depending on how regulatory environments progress in emerging scientific centers. However, we should do everything in our power to slow its emergence—the social implications of the technology if it develops prematurely is highly

troubling. Hopefully, given time, scientific regulation in these newer centers will grow more effective and begin to reflect the concerns in the west with regards to germ-line gene editing.

Appendix I – CRISPR/Cas9 Background

CRISPRs were first characterized by a group from the University of Osaka led by Yoshizumi Ishino, when he noticed that a bacterial DNA sequence he was studying was flanked by an unusual repetitive sequence[63]. However, a role for CRISPR/Cas9 in bacterial immune systems wasn't fully demonstrated until 2005, when a number of papers were published characterizing these loci of bacterial DNA as having an apparently extracellular origin[64]. These foreign "spacer" regions were surrounded by short repetitive segments of DNA, as Ishino described, and were eventually determined to have mostly originated from bacteriophages, a type of virus that infect bacteria. They were called clustered regularly interspaced short palindromic repeats, or CRISPRs.

The CRISPR/Cas9 system interferes with this process of viral replication by binding to viral DNA as soon as it is injected, before it has the opportunity to incorporate itself into cellular DNA. One region on the CRISPR/Cas system is responsible for recognizing and binding a specific piece of viral DNA. Another, the nuclease region, cuts the DNA once it has been recognized and bound. Because of the system's ability to recognize specific DNA sequences and to produce double stranded breaks in precise locations, CRISPR/Cas9 quickly gained recognition as a potential tool for genome editing.

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[63] Ishino Y, Shinagawa H, Makino K, Amemura M, Nakata A. "Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product". *Journal of Bacteriology,* 1987.

[64] Mojica FJ, Díez-Villaseñor C, García-Martínez J, Soria E "Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements". *Journal of Molecular Evolution*, 2005

Researchers first identify a DNA sequence of interest in their target cell and design a CRISPR RNA complimentary to some portion of that sequence. They can introduce CRISPR-coupled Cas9 system into a variety of cell types—including human cells—using simple techniques like electroporation, or more targeted techniques involving altered viruses[65]. Once the CRISPR/Cas9 is introduced into the cell it can be directed to produce a double-stranded break onto a specific region of host DNA, like a pair of scissors snipping through a thread[66]. In the simplest cases, this cutting action can be used to remove a sequence of DNA. If this approach is used, the cell will try to join the two cut ends of DNA back together by a process known as non-homologous end joining (NHEJ). This high-efficiency process usually introduces some number of random base insertions or deletions between the two ends, termed "indels[67]." The propensity for CRISPR/Cas9 to produce indels has been a major source of concern to researchers and regulator due to the tendency for cas9s to bind at off-target sites—a site not completely complementary to its guide RNA, but similar enough that the cas9 is able to bind for an instant—and thereby to introduce mutations into a random part of the genome that could lead to further disease[68]. The propensity for a particular CRISPR/Cas9 combination to bind to its intended target over all other targets is referred to as its fidelity.

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[65] Alberts, Bruce. Molecular Biology of the Cell, 5th Edition.
[66] Marraffini, Luciano A., and Erik J. Sontheimer. "CRISPR Interference: RNA-directed Adaptive Immunity in Bacteria and Archaea." Nature Reviews. Genetics. March 2010.
[67] Daniel P. Dever et al. CRISPR/Cas9 β-globin gene targeting in human haematopoietic stem cells. Nature. 2016 Nov 7
[68] Sander, J. D. & Joung, J. K. CRISPR–Cas systems for editing, regulating and targeting genomes. *Nature Biotechnol.* **32**, 347–355 (2014)

If coupled with the introduction of another DNA sequence with homologous ends, CRISPR/Cas9 can also be used to add in a new piece of DNA to the targeted sequence using homology-directed repair (HDR). Although this process does not typically produce the random indels seen in NHEJ, it occurs with a much lower frequency and therefore the efficiency of editing using HDR is much lower (i.e. fewer cells in a treated population will actually have their genomes successfully edited with the desired construct)[69].

CRISPR/Cas9 treatments generally have to optimize between both fidelity and efficiency. As the DNA binding region is made more specific to its correct target, it often binds less frequently to any target whatsoever; inversely, as efficiency increases and more cells treated are conferred with the desired genetic alteration, the amount of off-target activity tends to increase Various attempts at altering the nuclease to produce cas9 variants with improved fidelity and better efficiency are underway, however until they are more fully resolved these issues both increase the costs associated with developing safe CRISPR/cas9 treatments and limit the scenarios in which those treatments can be used[70].

HDR is considered to pose a somewhat lower indel risk, however the risk of off-target activity remains. The most significant advantage that HDR holds over NHEJ is that it presents the opportunity to actually introduce a new gene into the genome. This not only makes it a potentially useful laboratory tool for producing transgenic animal

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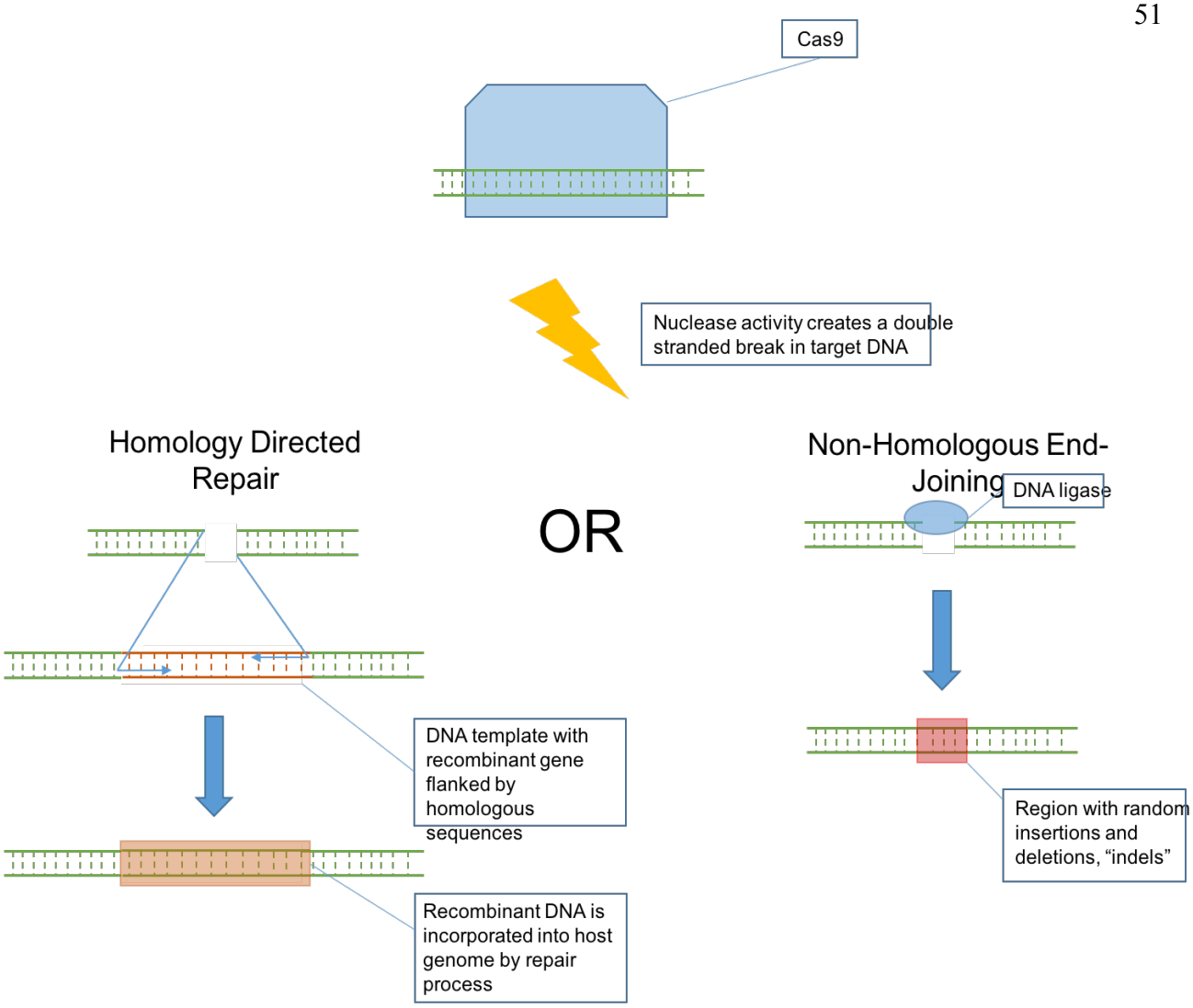
[69] Chu, Van Trung. "Increasing the Efficiency of Homology-directed Repair for CRISPR-Cas9-induced Precise Gene Editing in Mammalian Cells." *Nature Biotechnology* (2015

[70] Kleinstiver, Benjamin P., and Vikram Pattanayak. "High-fidelity CRISPR–Cas9 Nucleases with No Detectable Genome-wide Off-target Effects." *Nature News*. Nature Publishing Group, 06 Jan. 2016. Web. 01 Apr. 2017.

models, but also means that it could be used clinically to actually replace a malfunctioning gene with a functional copy, rather than simply excising the broken copy. This would allow function of the gene pathway to be restored and increases the number of diseases that CRISPR/Cas9 could potentially be used to combat. However, its low efficiency presents a potential problem; if the majority of cells are not affected by the process, clearly the therapeutic potential of the treatment is limited. Even so, efforts to improve the efficiency of HDR have had promising results, and ultimately the broader range of potential uses that accompany the ability to introduce new genes into the genome make HDR more attractive to many clinicians and scientists than NHEJ (though the latter process certainly has its uses).



Homology-directed repair can be induced by introducing a piece of target DNA sandwiched between two homologous ends—DNA regions complementary to the endogenous DNA on either side of the break. The cell's repair machinery will, with low frequency, mistake the target strand as the endogenous strand and ligate the two together. This must occur on both sides for the repair to be complete. In contrast, NHEJ simply requires that the broken sequences be ligated together, but produces random indels.

Although genome editing technology has existed for decades, only with the advent of CRISPR/Cas9 has it been so easily accessible to so many people. CRISPR/Cas9 distinguishes itself from older technologies in a number of ways. Its high adaptability and ease of use makes it particularly appealing compared to other (often higher-fidelity) options like zinc fingers and TALENs[71]. Although many treatments using these alternative technologies have already reached maturity, they are widely considered less promising than CRISPR/Cas systems due to the comparatively high cost of their development and the lack of ease in modifying them from one treatment to the next (although TALENs are somewhat cheaper to design that zinc fingers). Where Cas9 can simply be paired with a new guide RNA to target a new gene, an entirely new protein must be developed to change the DNA specificity of these other two technologies, which can be an extremely arduous and time-intensive process[72]. This ease of use and low cost barrier means that huge numbers of labs are able to create novel applications for the technology. In areas where regulatory bodies are less powerful or more lenient than others, this has resulted in the potential for the development of treatments with questionable ethical implications.

Clearly, CRISPR/Cas9 is a technology with a wide variety of potential uses. The ability to alter the genome at a cellular level has already shown itself to be hugely useful in a medical setting—treatments using TALENs and zinc finger caspases have been available for several years, and have seen considerable success[73]. A huge amount of

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[71] Yeadon, Ph.D Jim. "Pros and Cons of ZNFs, TALENs, and CRISPR/Cas." The Jackson Laboratory. N.p., Mar. 2014.
[72] Ibid
[73] Valton, Julien et al. "A Multidrug-resistant Engineered CAR T Cell for Allogeneic Combination Immunotherapy". Molecular Therapy, September 2015.

energy has been directed towards developing the technique to improve on its current limitations, and it has already shown great improvement in that regard since its discovery. Even though it is far from fully matured as a technology compared to alternatives like zinc fingers or TALENs, its simplicity of use combined with its high degree of flexibility makes it an extremely attractive prospect for use both in the lab and in the clinic.

Appendix II—Interview with Cliona Rooney

There are a number of prospective cancer treatments utilizing CRISPR/Cas9. In the Center for Cell and Gene Therapy at Baylor College of Medicine, Dr. Cliona Rooney's lab is developing T cells that have been genetically modified to recognize tumor antigens and improve survivability. In the near future, they hope to use these "CAR" (chimeric antigen receptor) T cells to improve survival in patients with certain types of cancer. I spoke to Dr. Rooney about these genetically modified T cells.

What are the goals of your research?

I am interested in creating T cell therapies to treat cancer. Most of these T cell therapies have got a lot of promise, but the problem that they face when infused into patients is that most tumors are very immunosuppressive, and they inhibit T cell proliferation, function, survival etc. So, we are always looking for ways to overcome that.

How do you use CRISPR/Cas9?

We have several projects in progress using the CRISPR system. Previously, we were able to introduce genes into cells but we did not have a simple way to remove genes. There were some ways – you could use TALENs, or a few others, but they are much more complicated than CRISPR.

Generally speaking, our projects focus on genetically enhancing T cells for cancer therapies. For example, we might introduce some gene that makes the T cells function better in fighting cancer cells. However, there is always the concern that the T cells will actually function *too* well, that they may start attacking the wrong targets or even become tumorigenic themselves. To combat this possibility, one of our projects is trying to create a suicide switch that would be active in our genetically engineered T cells. We want to have some way of turning off the T cells so that we can kill them if they proliferate too much in the patient. That way, if they are making the patient sick we can quickly stop the treatment. And quite often in T cell therapies, the T cells will produce too many cytokines in the patient -- this increases morbidity and even mortality, so we would really like to have some way of switching them off.

This modification also allows you to select *for* these T cells before you administer them to the patient. Since you've knocked out the salvage pathway, you can use a nucleoside analogue that will poison the salvage pathway and therefore kill off any unmodified T cells. Then after you infuse them, you can be sure that any cells you have administered will have your suicide gene in place in case it is needed. Knocking out HPRGT is one way we can use CRISPR/Cas in lymphocytes.

How else are you using CRISPR/Cas9?

Dr. Max Mamonkin is planning to use T cell therapy to treat patients with acute T cell lymphocytic leukemia. His strategy is to use a CAR that is specific for an antigen called CD7 expressed on TALL cells. But the problem is that the CAR T cells themselves express the same protein, so as soon as you produce them they start to target and kill each other. So his plan is to knockout CD7 in the T-cells using CRISPR/Cas. Then he will be able to insert the chimeric antigen receptor specific for CD7. The result is (hopefully) T cells that don't target each other, that grow and function perfectly normally, but still target TALL cells.

What are the major advantages to using CRISPR/Cas9 over other systems for gene modification?

There is lots of different ways to use it! You can do it much more quickly than any of the other systems out there – you just design the guide RNAs and order them, and two days later you can do your knockout [you can excise a gene from cells]. Peggy [Margaret] Goodell's lab developed this way to modify lymphocytes that's very effective, with very high efficiency. You can get over 90% knockout rate with very little toxicity to the T cells. Sometimes there is a bit of toxicity, but not much.

There is not nearly so much informatics involved, so it is also much cheaper. If we make a retrovirus vector to introduce a gene, we have to start with a producer cell line. If it is for clinical use, we have to do a huge amount of testing for all sorts of viruses, bacteria,

and just generally to make sure that the producer cells are exactly what we think they are. Just developing the producer cell line costs about a quarter of a million dollars, mostly because of the testing. Afterwards, you take the producer cell line and actually grow up the viral vector. You now have to test the vector's effects on your cell lines, whether it actually has the effect that you wanted. That costs another $250,000. So to make a viral vector for gene modification is about $500,000. Whereas CRISPR/Cas is much better, all you need to do is get the protein and make your clinical grade guide RNAs.

Don't you also have to worry about off-target effects?

We do… But you have to understand that this is different from using the system in vivo. We're using them on cells outside the body, so most of the off-target toxicities will be evident before implantation. We can do screens to survey whether they behave differently from normal T cells in ways that we don't expect, and detect other unintended effects that way. We make sure that they proliferate in response to antigen, that they don't proliferate when there is no antigen around, that they are killing the right targets, etc. Even so, so the risk to the patient is not non-existent. But these patients all have cancer, they've gone through several types of chemotherapy and other treatments and haven't shown much improvement. They'll have relapsed, probably multiple times, or they may be refractory to any known therapy. So they are probably going to die if they are not treated. In that situation, you can take more risk than with a patient who is

quite healthy, and who otherwise might not be at risk of dying any time soon. So the risk is justified – it is not that great of a risk in the end, and the patients are otherwise in a pretty desperate situation. And that's another reason that we want this suicide gene, so that if our T cells DO start behaving in unexpected ways we can halt their growth[74].

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[74] Rooney, Cliona. "Current Uses of CRISPR/Cas9 in the Baylor College of Medicine Center for Cell and Gene Therapy." March 2017.

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Joshua M. Brenner was born in Memphis, Tennessee on September 8th, 1994, and moved to Houston, Texas in 1998. He matriculated to the University of Texas at Austin in a neurobiology and Plan II dual degree in 2013. While at UT, he was editor-in-chief of UT's satirical magazine, the *Texas Travesty*, and studied axonal regeneration in the cerebellum with Dr. Hiroshi Nishiyama. He graduated Phi Beta Kappa in 2017 and plans to attend the University of California at San Francisco Neuroscience Graduate Program in the fall.